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Expression and X-Ray Crystallographic Analysis of the Recombinant Hemolytic Lectin CEL-III

Tomomitsu Hatakeyama*, Haruki Hidemura, Shuichiro Goda and Hideaki Unno
Department of Applied Chemistry, Faculty of Engineering,
Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan
**Tel: +81-95-819-2686, Fax: +81-95-819-2684, E-mail: thata@nagasaki-u.ac.jp*

Abstract

Recombinant hemolytic lectin CEL-III (rCEL-III) was expressed in *Escherichia coli* cells. Its hemolytic activity was much less than that of the native protein. X-ray crystallographic analysis of rCEL-III and native CEL-III (nCEL-III) revealed a slight difference in their tertiary structures, which may be caused by the amino acid replacements. It was inferred that these changes led to a decreased hemolytic activity of rCEL-III.

Introduction

The marine invertebrate *Cucumaria echinata* contains a Ca^{2+}-dependent, hemolytic lectin CEL-III, which exhibits strong hemolytic and cytotoxic activities by forming pores in the cell membranes (1, 2). The formation of membrane pores are mediated by oligomerization of its C-terminal domain (domain 3), while N-terminal domains (domains1 and 2), which show similarity with the ricin B-chain, bind cell surface carbohydrate chains, thereby facilitating interaction of domain 3 with target cell membranes (3). In this study, the recombinant CEL-III (rCEL-III) was expressed in *E. coli* cells using previously obtained cDNA, and its three-dimensional structure was solved by X-ray crystallographic analysis.

Experimental

rCEL-III was expressed in BL21(DE3)pLysS cells using previously obtained cDNA, inserted into pET-3a vector. Crystallization of the protein was done by mixing the protein solution (7 mg/ml, 2-4 μl) with the same amount of the reservoir solution (12% (w/v) polyethylene glycol 8000, 100 mM bis(2-hydroxyethyl)imino-tris(hydroxymethyl)-methane/NaOH, pH 6.5, and 200 mM magnesium acetate), and subjecting it to sitting drop vapor diffusion at 20°C. Diffraction images from the crystals were collected using synchrotron radiation at the Photon Factory (Tsukuba).

Results and Discussions

The resulting recombinant protein exhibited much less hemolytic activity (1/100 compared with nCEL-III) (Fig. 1). X-ray diffractions of the rCEL-III crystals were observed up to 2.08 Å, and its tertiary structure was solved by the molecular replacement method using nCEL-III (Fig. 2) (4) as a search model. In the structure of rCEL-III, there are 14 differences in amino acid residues, which might be due to microheterogeneity of the gene or mutations introduced during cloning steps using PCR. The main chain structures of rCEL-III and nCEL-III also showed a slight difference in the distance between domains 1 and 3. It was inferred that these changes led to a marked decrease in the hemolytic activity of rCEL-III.

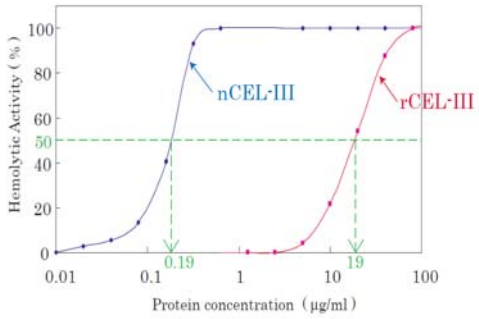

Fig. 1. Hemolytic activity of rCEL-III and nCEL-III.

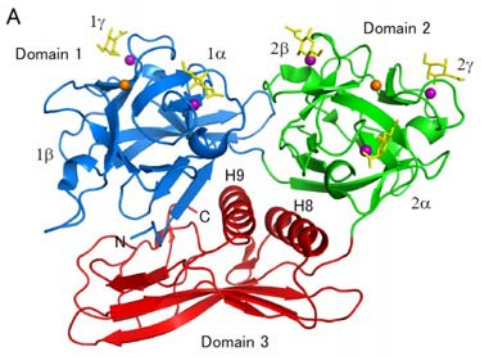

Fig. 2. Tertiary structure of CEL-III with bound sugars (yellow).

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